

**Amendments to the Specification:**

Please replace the paragraphs from page 25, line 11 through p.35, line 28 with the following amended paragraphs:

Example 1. Synthesis of N-Isopropylhydroxylamine. Acetic acid (10.8 g) was added to a cooled solution of 2-nitropropane (5.35 g) and zinc dust (5.89 g) in 95% ethanol (350 mL) at such a rate to maintain the temperature below ~~10.degree. C~~ 10 °C. The reaction was stirred for three hours and the solvent removed in ~~vacua~~ vacuo. The residue was extracted three times with dichloromethane. The combined extracts were dried over magnesium sulfate, filtered, and solvent stripped. The crude hydroxylamine product was used without further purification. Other hydroxylamines may also be prepared by this procedure.

Example 2. Synthesis of Hydroxylamines By Reduction of Oximes. Various hydroxylamines were prepared according to the procedures of R. F. Borch et al., J. Amer. Chem. Soc., 1971, 93(3):2897 from the corresponding oxime. Specifically, a 3-necked round bottom flask equipped with a stirring motor, a pH meter probe and an addition funnel is charged with a solution of the oxime in methanol (ca. 0.4M). To the stirring solution is added 0.68 equivalents of NaBH<sub>3</sub> CN in portions. The addition funnel is filled with 4M HCl in MeOH. The amount of the acid solution prepared should be roughly 3/4 of the volume of MeOH used to dissolve the oxime. The HCl solution is then added slowly to the oxime until pH comes down to about 4 and stabilizes at that value. The solution is then allowed to stir at ambient temperature for ca. 4 hours. HCl is added as necessary to keep the pH at 4. (A small sample can be periodically removed and worked-up to determine if the reaction is complete). When the reaction is complete, the solution is decanted into a 1-necked round bottom flask and MeOH is removed in vacua. (While removing the methanol by rotoevaporation, the solvent trap should be filled with NaOH (1 eq.) to quench HCN stripped off with MeOH). After the methanol has been removed, the residue is dissolved in water and extracted with methylene chloride (~~4-times~~ 4 times). The organic phases are combined, dried over MgSO<sub>4</sub> and stripped to dryness to provide the hydroxylamine product (as determined by NMR and DSC).

Example 3. Synthesis of N-Cyclohexylhydroxylamine cyclohexylhydroxylamine. N-Cyclohexylhydroxylamine cyclohexylhydroxylamine hydrochloride (commercially available from Aldrich, 1001 West Saint Paul Avenue, Milwaukee, Wis. 53233 U.S.A.) was suspended in ether (about 200 mL of ether for 6 grams of the hydroxylamine salt) and extracted three times with 5% NaOH in brine. The organic phase (white fluffy crystals of N-cyclohexylhydroxylamine suspended in ether) was transferred to a round bottom flask and the ether was removed in vacuo. The resulting crystals were dried under a high vacuum for about 20 min. to afford the title compound.

Example 4. Treatment of Acute CNS Disorders. In this example, the ability of subject hydroxylamines to reduce the infarct volume in an in vivo stroke model is demonstrated. A rat permanent middle cerebral artery occlusion (MCAO) model is used to determine stroke treatment efficacy. MCAO is a representative model of acute CNS disorders. See, for example, M. D. Ginsberg et al., "Rodent Models of Cerebral Ischemia" (1989) Stroke, 20:1627-1642. In this stroke model, the middle cerebral artery is permanently occluded via cauterization to produce a focal stroke. The hydroxylamines are then administered as a 10 mg/kg i.v. bolus dose three hours post MCAO through a catheter surgically implanted in the jugular vein. Two days post MCAO, the rats are sacrificed and the extent of brain damage assessed using tetrazolium staining (TTC staining) followed by computer image analysis to quantitate infarct volumes, i.e., the regions of dead tissue. The mean infarct volume for rats treated with the test compound is significantly less than the mean infarct volume for control rats not treated with the hydroxylamine. Thus, the hydroxylamines can reduce the mean infarct volume of a stroke when administered three hours post stroke compared to controls.

Example 5. Inhibition of A $\beta$  Beta-Pleated Sheet Formation. The deposition of amyloid  $\beta$ -peptide (A $\beta$ ) is associated with the development of Alzheimer's disease. See, for example, G. G. Glenner et al. (1984) Biochem. Biophys. Res. Commun., 120:885-890; and R. E. Tanzi (1989) Ann. Med., 21:91-94. Accordingly, compounds which effectively disrupt the formation of A $\beta$ (1-40) or A $\beta$ (1-42) beta-pleated sheets are potentially useful for preventing and/or reversing such amyloid deposits. Thioflavin T (ThT) is known to rapidly associate with

beta-pleated sheets, particularly the aggregated fibrils of synthetic A $\beta$ (1-40). This association gives rise to a new excitation maximum at 440 nm and to enhanced emission at 490 nm. In this experiment, the ability of the subject hydroxylamines to inhibit the association of ThT with synthetic A $\beta$ (1-40) or A $\beta$ (1-42) is demonstrated by measuring changes in fluorescence.

The experiments are performed using a CytoFluor II fluorescence plate reader having the following parameters: Filters (Excitation and Emission) = 440 nm/20 and 490 nm/40; Gain = 75; Cycle to Cycle Time = 30 min; Run Time = 720 min (24 cycles) or dependent on exp. design; Plate = 96 well. Into each well is aliquoted 95  $\mu$ L of ThT (3  $\mu$ M) prepared in PBS (pH 6.0), 2  $\mu$ L of the compound to be tested (10  $\mu$ M) prepared with 0.05% of methylcellulose in PBS (pH 6.0), and 3  $\mu$ L of A $\beta$ (1-40)(3  $\mu$ g) prepared with dH<sub>2</sub>O. The fluorescence measurement begins when the A $\beta$ (1-40) is added and continues for a total of 12 hours. The percent inhibition of beta-pleated sheet formation is calculated from the relative fluorescence unit difference between aggregation in the presence and in the absence of the test compounds. The data show that compounds prepared in Examples 1, 2 and 3 above inhibit A $\beta$ (1-40) beta-pleated sheet formation compared to the controls. In experiments conducted in a similar manner using A $\beta$ (1-42) instead of A $\beta$ (140), the compounds similarly ~~inhibited~~ inhibit A $\beta$ (1-42) beta-pleated sheet formation compared to the controls.

Example 6. Protection Against A $\beta$ (2535)-Induced Neuronal Cell Loss. Patients with Alzheimer's disease are known to suffer a progressive loss of neuronal cells. See, for example, P. J. Whitehouse Whitehouse et al., (1982) Science, 215:1237-1239. This experiment demonstrates the ability of subject hydroxylamines to protect against A $\beta$ (25-35)-induced neuronal cell loss. Sprague Dawley rat hippocampus of 18-day-gestation embryos are excised and then dissociated by trituration to prepare primary neuronal/astrocyte cultures. Cells (3 x10) are plated on 35 mm poly-D-lysine-coated plates containing Eagle's minimum essential medium supplemented with 10% fetal bovine serum. After 3-5 hours, the original medium is removed and replaced with 1 mL of fresh medium. Cultures are maintained at 37°C. in a 5% CO<sub>2</sub>/95% air humidified incubator.

To the cells (7 DIV) is added 30  $\mu$ M of A $\beta$ (25-35) dissolved in dH<sub>2</sub>O (stored at -20°C.) and 100  $\mu$ M of a test compound (e.g., a compound of Example 1, 2 and 3 above) in 1% methylcellulose. Controls are also conducted without the test compound. The percentage of morphologically viable neurons is determined counting the number of viable neurons after 96 hours treatment compared to the number of neurons before treatment in the same premarked culture regions (three regions/culture, n=6). The data show that the hydroxylamines ~~reduced~~ reduce A $\beta$ (25-35)-induced neuronal cell loss compared to the controls. In experiments conducted in a similar manner using A $\beta$ (1-40) instead of A $\beta$ (25-35), the compounds prepared in Example 1-3 above also reduce A $\beta$ (1-40)-induced neuronal cell loss compared to the controls.

Example 7. Reduction of Inflammation. In Alzheimer's disease, stroke and multiple sclerosis, researchers have implicated an inflammatory response in the etiology of the disease. See, for example, P. S. Aisen et al., (1994) Am. J. Psychiatry, 151:1105-1113; D. W. Dickson et al., (1993) Glia, 7:75-83; and S. D. Yan et al., Proc. Natl. Acad. Sci. USA, 94, 5296 (1997). This response has been modeled in cell culture by utilizing various factors to simulate the inflammatory response. Such factors include lipopolysaccharide (LPS), an agent known to cause the expression of nitric oxide and other cytokines; and interferon  $\gamma$  (INF- $\gamma$ ), another agent implicated in the inflammatory/cytokine response. This example demonstrates the ability of subject hydroxylamines to reduce the inflammation caused by LPS and INF- $\gamma$ . In this experiment, the cell culture system is composed of E16 rat pure cortical neuronal cells (treated with 10  $\mu$ M Ara C to retard astrocyte growth) that are plated on a confluent bed of two week old cortical glial cells prepared from the cortices of 1 day old rat pups and allowed to grow for one week. To these cells is added LPS (20  $\mu$ g/mL), IL-1 $\beta$  (40 mg/pg/mL), and INF- $\gamma$  (200 U/mL), either with or without 100  $\mu$ M of the test hydroxylamine. Two days later, cell viability ~~was~~ is assessed using the lactate dehydrogenase (LDH) assay to monitor cytosolic protein leakage due to cell membrane damage. The results show that the hydroxylamines ~~reduced~~ reduce the inflammation caused by LPS and INF- $\gamma$  compared to the control.

Example 8. Reduction of  $\beta$ -Amyloid-Induced Increased Cytokine Release. This experiment demonstrates the ability of the hydroxylamines to reduce the  $\beta$ -amyloid-induced

increased release of cytokines, such as interleukin-1 $\beta$  (IL-1). THP-1 cells, a human monocyte cell line from American Type Culture Collection, are grown in RPMI-1640 medium plus 10% fetal bovine serum (FBS, not heat-inactivated) in T-flasks. The medium is changed every two days by spinning down (800 rpm, 5 minutes) the cells and ~~added~~ adding the same fresh medium. Alternatively, the cultures are maintained by the addition of fresh medium. The cultures are maintained at a cell concentration ranging from between  $1 \times 10^5$  and  $1 \times 10^6$  cells/mL. Because sera may contain unknown factors which can affect macrophage/monocyte IL-1 production, the FBS is reduced to 5% for 24 hours. The FBS is further reduced to 2% over two days prior to starting each experiment. The cells are collected by centrifugation and resuspended to 2% FBS. Cell numbers are calculated and cells plated on 24-well plates ( $3 \times 10^5$  cells/0.6 mL/well). Cells are then treated with LPS (0.5  $\mu$ g/ml or 0-10  $\mu$ g/ml for LPS dose-response experiments) alone or in combination with A $\beta$  peptides (5  $\mu$ M or 0.05-5  $\mu$ M for dose-response experiments). When determining the effect of the hydroxylamines on cytokine release, 100  $\mu$ M of the hydroxylamine is added with the LPS and A $\beta$ 25-35, and this mixture incubated for 48 hours prior to performing ELISA.

IL-1 $\beta$  secretions into medium by LPS-stimulated THP-1 cells, in the presence or absence of amyloid peptides and a test compound, are assayed with a commercially available ELISA kit (R & D Systems). Briefly, a microtiter plate coated with a murine monoclonal antibody to human IL-1 $\beta$  is supplied by the manufacturer. Standards and samples are pipetted into the wells and any IL-1 $\beta$  present bound by the immobilized antibody. Unbound proteins are washed away, and a horseradish peroxidase-linked polyclonal antibody specific for IL-1 $\beta$  added to the wells to "sandwich" the IL-1 $\beta$  bound in the initial step. After washing to remove any unbound antibody-enzyme reagent, a substrate solution (1:1 hydrogen peroxide--tetramethylbenzidine, v/v) is added to the wells, and color developed in proportion to the amount of IL-1 $\beta$  bound in the initial step. Color development is stopped with 2N sulfuric acid, and the optical density of the standard and the test samples measured at 450 nm. The amounts of IL-1 $\beta$  present in the samples are calculated based upon a standard curve. Assays are run in quadruplicate wells. The data show

that the hydroxylamines reduce the  $\beta$ -amyloid-induced increased release of interleukin-1 $\beta$  compared to the controls.

Example 9. Reduction of Locomotor Impairment Due to A $\beta$ -Peptide. This experiment demonstrates the ability of the hydroxylamines to reduce the in vivo impairment of animals treated with A $\beta$ -peptide. Male Sprague-Dawley rats (250-400 g) are given an ipsilateral injection of 20  $\mu$ g of A $\beta$ (25-35) into their substantia nigra. Prior to the injection, the rats are fasted overnight, and then each ~~received~~ receive an oral treatment of the hydroxylamines (prepared in Examples 1, 2 and 3 above, 10-100 mg/kg) dissolved in aqueous 1% methyl cellulose or the vehicle alone, one hour before and three hours post the A $\beta$ -peptide stereotaxic injection. One week after treatment, the rats are dosed s.c. with 0.5 mg/kg apomorphine (dissolved in 0.1% vitamin C in isotonic saline) and the circling reflex monitored using a Rotorat computerized behavioral monitoring apparatus for the time period between 15 and 30 minutes of being placed in the arena. Impairment of the animals due to A $\beta$ -peptide is determined by measuring the number of rotations over the 15 minute period. A higher number of rotations per period indicates more physical impairment. The results show that the hydroxylamines ~~reduced~~ reduce the number of rotations per period and hence, the locomotor impairment, of rats injected with A $\beta$ (25-35) compared to A $\beta$ (25-35)-treated controls.

Example 10. Reduction of Spatial Learning Deficit. This experiment demonstrates the ability of the hydroxylamines to reduce spatial learning deficiencies in vivo. Treatment of rats with N-nitro-L-arginine, a nitric oxide synthase inhibitor, is known to cause a deficit in spatial learning. See, for example, G. A. Bohme et al., (1993) PNAS, 90:9191-9194. Rats treated with N-nitro-L-arginine wander aimlessly throughout their enclosure whereas untreated rats spend most of their time in the quadrant in which they are initially placed and stay away from the open area in the middle of the enclosure. This N-nitro-L-arginine-induced spatial learning deficit is used as a model for learning deficits caused by Alzheimer's disease and other dementias. In this experiment, 10 mg/kg of a hydroxylamine or a control are administered 30 min before each of nine doses of N-nitro-L-arginine (100 mg/kg. iip.). The results show that rats dosed with N-nitro-L-arginine wander equally around the perimeter of the enclosure and readily cross the

center of the field. In contrast, rats treated with the hydroxylamines show a preference for the area of the enclosure into which they ~~were~~ are first placed and rarely cross the center of the enclosure. This behavior is essentially the same as rats treated with a saline control (i.e., without N-nitro-L-arginine). These results demonstrate that the hydroxylamines prevent the spatial learning deficit caused by N-nitro-L-arginine.

Example 11. Prevention of MBP-Induced Experimental Allergic Encephalomyelitis. ~~Multiple sclerosis (MS) is a chronic inflammatory CNS disorder caused by demyelination in the brain and spinal cord. The disease is characterized by progressive CNS dysfunction, including muscular weakness, tremor, incontinence, ocular disturbances, and mental dysfunction, with remissions and exacerbations. At present, the only treatment for MS is physical therapy.~~

Experimental allergic encephalomyelitis (EAE) induced by injection of myelin basic protein (MBP) or MBP peptide fragments is reported to be a useful model for MS multiple sclerosis. See, for example, D. E. McFarlin et al., "Recurrent Experimental Allergic Encephalomyelitis in the Lewis Rat," The Journal of Immunology, 113(2): 712-715 (1974). This experiment demonstrates the ability of the hydroxylamines to prevent MBP-induced EAE.

Acclimated female Lewis ~~rats, rats~~ (Harlan; 200-250 g) are used in this experiment since this strain of rat is genetically highly susceptible to EAE. In the experiment, 100 mg/kg of the hydroxylamines (prepared in Examples 1, 2 and 3 above) or a vehicle alone (control) is are administered po once a day from days 4 to 18. On day 1, the rats receive an injection of 100  $\mu$ g of MBP peptide, from guinea pig brain, plus 500  $\mu$ g of H37RA Mycobacterium in 0.10 ml complete Freund's adjuvant divided equally between the two hind foot-pads.

The rats are evaluated on a 0-6 scale every day after day 7 until day 18 (effects usually begin day 10 and peak day 15). See E. Heber-Katz, "The Ups and Downs of EAE," International Reviews Immunology, 9: 277-285 (1992). These results show that the hydroxylamines completely ~~counteracted~~ counteract the effect of MBP in this test.

Example 12. Prevention of Weight Loss. Animals exposed to MBP or MBP peptide exhibit significant weight loss as compared to controls exposed to Freund's adjuvant alone. To determine if the hydroxylamines prevented such weight loss, the animals in the EAE model

described in Examples 1, 2 and 3 above ~~were~~ are weighed daily. The results show that those animals receiving the hydroxylamines exhibit normal or above normal weight gain, whereas the animals receiving MBP without the hydroxylamines showed serious weight loss.

Example 13. Reduction of Learning Deficit in Autoimmune Mice. This experiment demonstrates the ability of the hydroxylamines to reduce learning deficiencies in autoimmune mice. Male MRL/MpJ controls and Fas mutation mice were either dosed orally with 1% methylcellulose ("~~MC~~") or with 100 mg/kg of the hydroxylamines (prepared in Examples 1, 2 and 3 above, "~~test compound~~") in 1% methylcellulose for 9-10 weeks. Following dosing, animals of approximately 4 months of age are tested in an active avoidance T-maze. In the one day test, animals are analyzed for acquisition to avoid shock within the first five trials of the test. The data reveal that animals administered the hydroxylamines show a 50% protection in acquisition learning deficit compared to Fas mutated animals receiving only 1% methylcellulose.

Example 14. Comparing in vivo the efficacy of subject hydroxylamines, PBN, and two monosulfonate PBN compounds as agents for protecting against neuron loss following brain ischemia and reperfusion injury. The test procedure is that reported by W. Cao, J. M. Carney, A. Duchon, R. A. Floyd and M. Chevion as "Oxygen free radical involvement in ischemia and reperfusion injury to ~~brain~~ brain", Neuroscience Letters, 88 (1988), 233. In the experiments a test compound is administered to groups of six gerbils i.p. as a single dose 30 min before 5 min bilateral carotid occlusion. The density of neuronal nuclei in a 100 micron is measured. Two controls are present--controls which receive no test compound and controls which receive no test compound and no brain ischemia. The compounds of the invention show advantages as compared to the prior art compounds. These results show a clear increase in potency for neural protection for the subject hydroxylamines compared to PBN and two closely related analogs, and less ~~in~~ toxicity compared to PBN.

Example 15. Comparing the subject hydroxylamines to PBN and two sulfonate analogs in post-ischemia treatment. The general method described above is used but the test compounds are administered i.p. as a single dose 30 min after reperfusion following 5 min ischemia. The



results show that the subject hydroxylamines are again more potent at low doses, and more potent and less toxic at high doses.

Example 16. Comparing the subject hydroxylamines with PBN to determine the relative effectiveness for protection of neuronal loss when administered i.v. 60 min after reperfusion onset following 5 min ischemia in gerbils using the general test method described above. The results illustrate that the subject hydroxylamines are of significantly greater therapeutic benefit than is PBN in a clinical treatment setting following injury to the brain. Neither PBN nor the subject hydroxylamines have an effect on neuronal density in control gerbils without brain injury.

Example 17. Brain injury can manifest itself as behavioral changes. In this experiment, young adult (3-4 months of age) gerbils are tested to determine their ability to perform an 8-arm maze test 24 hours following an ischemic event as described above. As compared to nonischemic animals, when untreated they ~~committed~~ commit many more errors. PBN and subject hydroxylamines are administered to some of the test animals. Gerbils treated with high doses of the hydroxylamines have error levels indistinguishable from those of nonischemic animals. PBN is less effective. This shows that subject hydroxylamines can protect against the loss of temporal/spatial short term memory following ischemia (24 hours post) errors in 8-arm radial maze test of young gerbils following 5 min ischemia.

Example 18. The ability of the subject hydroxylamines to reduce infarct volume following an ischemic event. While PBN and the hydroxylamines are both effective at low doses, at high doses hydroxylamines ~~gave~~ give the best protection and PBN ~~was~~ is toxic.

Example 19. In this study, subject hydroxylamines and PBN are compared for their ability to impart lethality protection (% survived) in aged gerbils (18-24 months of age, n=12/group) from 10 min ischemia when given 30 min before ischemia. The hydroxylamines are superior at all dose levels, and achieve complete protection at high levels while PBN is only partially effective.

Example 20. An important advantage of the subject hydroxylamines as compared to PBN, ~~is its~~ are their markedly diminished toxicity. Acute lethality in C57BL/6L mice is

determined based upon varying sizes of single i.p. doses of hydroxylamine. PBN shows significant toxicity at 560 mg/kg dose levels. The hydroxylamines show no toxicity at doses nearly twenty times as great.

Example 21. Another undesirable systemic effect which has been observed in vivo with nitron radical traps is a depression in body temperature. This toxicity can have serious health consequences and also can complicate diagnosis of other conditions. The subject hydroxylamines are administered to mice and gerbils at levels as high as 1000 mg/kg with no measurable temperature decrease. In contrast, PBN gives up to an 8°C decrease in body temperature at a ~~does~~ dose of only 500 mg/kg.

Example 22. Hydroxylamines effectiveness in the treatment of conditions characterized by protracted low grade oxidative stress upon the central nervous system and gradual progressive central nervous system function loss by effectiveness in a model for Alzheimer's disease ("AD"). Studies have demonstrated that there is an age-associated increase in protein oxidation and loss of enzyme activities in the brain of aged individuals. Tissue cultures of fibroblasts from aged individuals and red blood cells of different ages both show an exponential increase in protein carbonyl content (a measure of protein oxidation) and a decrease in marker enzyme activities. Brain protein oxidation progressively increases over the life span of the individual. The role of abnormal amyloid precursor peptide processing and metabolism in AD has also been explored in a number of different models. In vitro studies using embryonic hippocampal neuronal and neuronal/glia cultures have demonstrated that  $\beta$ AP 1-40 produces cytotoxicity over an extended period of co-incubation. When this peptide is infused into rat brains, lesions are produced. Some of the proposed breakdown fragments of  $\beta$ AP are also neurotoxic, e.g. PAP (25-35). The neurotoxicity appears to be both mediated via glutamate receptors, and also by non-glutamate receptors mechanisms. Confocal microscopy studies of neuronal cultures have demonstrated that exposure to  $\beta$ AP (1-40) results in oxidative ~~stress-~~ stress.

It has been demonstrated that  $\beta$ AP fragments can directly inactivate glutamine synthetase (GS) and creatine kinase (CK) in tissue extracts and in cultured hippocampal neurons and glia. While the hydroxylamines and PBN each show the ability to protect GS and CK against the

effects of  $\beta$ AP fragments, the hydroxylamines WILL give complete protection, and in fact can at least partly reverse the effects of oxidation. In contrast, PBN's effectiveness is quite limited as it asymptotically levels out at a substantially incomplete level of protection.

Example 23. The effectiveness of the subject hydroxylamines in preventing central nervous system damage caused by stroke. Rat focal ischemia results show the efficacy of subject hydroxylamines in a rat focal ischemia model. In this model, Sprague Dawley rats (200-300 g) undergo a permanent middle cerebral artery occlusion (MCAO) to induce a focal stroke. Subject hydroxylamines are administered after the permanent occlusion as first an intraperitoneal (i.p.) bolus dose and then by intravenous (i.v.) continuous infusion during the remaining time up to 24 hours post stroke. The doses ~~used were~~ are either 100 mg/kg, i.p., followed by 4.2 mg/kg/hr, i.v., or 10 mg/kg, i.p., followed by 0.42 mg/kg/hr, i.v. The rats are sacrificed 3 days post stroke, the tissue processed histologically using triphenyltetrazolium staining techniques, and the infarct volume, the area of total cell necrosis, quantitated using image analysis. The results of these experiments demonstrate that subject hydroxylamines provide significant protection, approximately 70%.

Example 24. Evaluating the ability of the subject hydroxylamines to ameliorate oxidation-caused side effects of anticancer therapy. Adriamycin is a widely used anticancer agent. It is known to be very effective, but it is also known to have serious side effects arising from its tendency to cause oxidative damage. These side effects include causing serious levels of cardiac damage at high dose levels. These side effects have often limited the use of this agent or limited the dose levels that can be employed to levels which are below those desired for maximum antineoplastic disease effectiveness.

Experiments demonstrate that the subject hydroxylamines are effective at reducing the side effects of anticancer agents such as adriamycin, and permitting higher dose levels of adriamycin to be tolerated by animals. C57BL/6J and DBA/2J male mice (35-40 g) are tested for the acute lethal effects of adriamycin, and the prevention of acute lethality by pretreatment doses of subject hydroxylamines. Mice are injected i.p with either saline or subject hydroxylamines 30 minutes prior to administration of adriamycin. The acute lethality of adriamycin ranges from 10

to 30 mg/kg. The LD<sub>50</sub> for adriamycin ~~in these tests~~ was found to be 25 mg/kg in both mouse strains. Hydroxylamines doses up to 300 mg/kg, without adriamycin treatment, have no effect on survival in the two mouse strains. Pretreatment with 30 and 100 mg/kg of hydroxylamines produces dose related shifts in the adriamycin lethality dose effect curve. A dose of 100 mg/kg of subject hydroxylamines produces a 5-fold shift to the right (in the direction of reduced lethality). Thus, the combination of subject hydroxylamines with adriamycin results in a marked increase in the maximally tolerated dose. These higher doses are in the range that would effectively kill multi-drug resistant tumors.

Comparative Tests. PBN pretreatment results in a slight shift to the right in the adriamycin dose-effect curve. While the subject hydroxylamine dosages can be increased to 300 mg/kg in combination with adriamycin, there is an upper limit for this combination with PBN. A dose of PBN of 100 mg/kg produces slight sedation, and 300 mg/kg ~~yielded~~ yields marked sedation and some combined toxicity (10-20% lethality). Hydroxylamines/adriamycin does not produce any combined toxicity at doses of hydroxylamine of up to 300 mg/kg.

Example 25. Safety Testing. The subject hydroxylamines and PBN are tested for their acute toxicity in male Sprague Dawley (200-300 g) rats. The compounds are administered at 1000 mg/kg, i.p., to groups of 6 rats. After 3 days lethality is assessed. Hydroxylamines causes no lethality, while PBN ~~is lethal to 5 of the 6 rats used~~ causes lethality in this test. These data confirm the gerbil data in that the hydroxylamines have higher safety than PBN.